REF 1995-061

Arch. Environ. Contam. Toxicol. 28, 519–523 (1995)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Regressors of Whole-Carcass Zinc Phosphide/Phosphine Residues in Voles: Indirect Evidence of Low Hazards to Predators/Scavengers

R. T. Sterner, R. E. Mauldin

U. S. Department of Agriculture, Denver Wildlife Research Center, Bldg. 16, Federal Center, Denver, Colorado 80225-0266, USA

Received: 1 August 1994/Revised: 28 October 1994

Abstract. Whole-carcass residues of the rodenticide zinc phosphide (Zn_3P_2) and hydrolyzed phosphine (PH_3) were determined for voles (*Microtus spp.*) that died following ingestion of a 2% Zn_3P_2 steam rolled oat (SRO) groats bait. Procedures involved: a three-day acceptance test to assess vole consumption (n = 27) of control SRO groats and several one-day Zn_3P_2– (n = 13) or control-bait (n = 4) tests to characterize onset of pharmacotoxic signs and to obtain fatally-dosed carcasses for residue analyses. Carcasses were stored in liquid nitrogen (LN_2) to maximize retention of Zn_3P_2/PH_3 residues prior to chemical determinations. Linear regressions were computed between pairs of consumption and residue variables. Main results were the following: (1) mean (±SD) consumption of control bait was 2.5 (±0.9), 3.0 (±0.9), and 2.8 (±0.8) g on days 1, 2, and 3, respectively (≥10.6 ± 4.6% of body weight); (2) all test-bait voles (n = 13) died ~4–12 h after bait presentation, with lethargy and respiratory distress key signs of toxicosis; (3) whole-carcass Zn_3P_2 residues averaged 1.73 mg (min-max: 0.31–4.95), and PH_3 residues averaged 10.6 μg (min-max: 0.5–21.0); and (4) significant linear regressions were found between bait consumption/Zn_3P_2 intake and body weight ($r^2 = 0.64$, $p \leq 0.001$), carcass Zn_3P_2 and bait consumption/Zn_3P_2 intake ($r^2 = 0.32$, $p \leq 0.043$), and carcass Zn_3P_2 and body weight ($r^2 = 0.60$, $p \leq 0.002$). Certain analytical and hazards issues are discussed.

Zinc phosphide is an acute rodenticide that has widespread agricultural and public health applications (Marsh 1988; Gratz 1973). One registered agricultural use is to control vole populations in orchards (reduce the girdling, debarking of trees). Additionally, recent outbreaks of Hantavirus Pulmonary Syndrome (HPS) demonstrate the continued need for effective, acute rodenticides to control disease vectors (Childs *et al.* 1994). Acute-oral, median-lethal-dose (LD_{50}) values for Zn_3P_2 in meadow and prairie voles are 15.7–18.0 and 16.2 mg/kg, respectively (Bell 1972; Hood 1972).

Correspondence to: R. T. Sterner

Few direct or indirect studies have assessed potential primary (exposed or undigested bait) or secondary (tissue residues) hazards of Zn_3P_2-dosed rodents to nontarget predators/scavengers (Bell and Dimmick 1975; Tkadlec and Rychnovsky 1990). Direct studies refer to predator/scavenger ingestion of Zn_3P_2-/PH_3-dosed rodent carcasses. Indirect studies refer to carcass estimates of Zn_3P_2/PH_3 residues only. A literature search yielded only one report of available PH_3 residues in Zn_3P_2-baited-rodent carcasses, with few quantitative data presented (Tabata 1986). Estimates of whole-carcass Zn_3P_2 and PH_3 residues present in fatally-dosed voles afford predictions of potential hazards.

This report describes development of cryogenic-preservation and gas-chromatographic (GC) techniques for estimating whole-carcass Zn_3P_2/PH_3 residues in a mixed sample of meadow (*M. pennsylvanicus*) and prairie voles (*M. ochrogaster*). Linear relationships between selected ingestion/weight/residue variables are also summarized.

Materials and Methods

Zinc Phosphide

The Zn_3P_2 technical product (CAS No. 1314-84-7) was obtained from the Pocatello Supply Depot, Pocatello, ID, which had obtained it from Gallard-Schlesinger Industries, Inc., Carle Place, NY. The product was a gray-black powder. Mean (±SD) pre-/post-study analyses showed that the technical Zn_3P_2 concentrations were 94.5% (±1.1) and 94.4% (±3.9), respectively.

Voles

A total of 31 voles were purchased from Genesis Laboratories, Inc, Ft. Collins, CO. All voles were live-trapped near Colgate, WI, and were air-freighted to Denver after a ~one month capture/hold period. Upon arrival at the Denver Wildlife Research Center (DWRC), voles were dusted with a commercial flea and tick powder, examined, weighed, numbered, and quarantined for 18 days.

Voles were housed in a temperature-regulated room (18–28°C min-max), with a 0600–1800:1800–0600 h light:dark cycle. They were

kept individually in disposable polystyrene cages (32.5 × 23 × 12.5 cm) having stainless-steel-wire tops; cages were replaced every 14–21 days. Non-toxic wood shavings covered the cage bottoms. Except during tests, voles were maintained *ad libitum* on Purina® Laboratory Rodent Chow Pellets® (Purina Mills Inc, St. Louis, MO)[1] and water; two slices of fresh carrot were also provided daily.

Acceptance Test (Control-bait)

A three-day, single-choice, free-feeding test was conducted to determine the acceptance of SRO groats plus vehicle by the voles.

Voles: Twenty-seven voles were used. These were composed of 17 prairie voles (8♂ 9♀), five meadow voles (3♂ 2♀), and five voles of "unidentified" species/gender.

Baits: A 2,000 g batch of control (0.0% Zn_3P_2) bait was prepared by mixing 2% (wt/wt) of Alcolec-S (American Lecithin Co., Woodside, NY) with mineral oil (81:19) and 98% SRO groats (LaCrosse Milling, Cochrane, WI). Baits were sealed in plastic bags and frozen until use.

Materials: Control bait was presented to each vole in a glass dish (10-cm diameter × 3.25-cm deep); a stainless-steel disk (7-cm diameter having 12 1.4-cm-diameter holes) was placed on top of the bait to reduce spillage.

To measure spillage, wood shavings were replaced with absorbent paper (~14 × 22 cm). A honeycombed white plastic (~13 × 21.6 × 1 cm with 1.3-cm^2 holes) insert covered the paper; this elevated each vole and allowed recovery of spilled particles.

Two identical sets of cages, food dishes, and water bottles were set up and labeled. During the test, voles were switched between alternate cages daily.

Procedures: Each animal was weighed (nearest 0.01 g) using an electronic digital balance (Mettler Instrument Corp., Hightstown, NJ; Mdl. PE-3600) before and after the test. Voles were fasted ~16 h prior to the initial bait exposure.

On day 1 (~0700 h), the control bait was thawed and ~15 g quantities were weighed into respective dishes (nearest 0.01 g). At ~0800 h, the voles, pre-weighed dishes, and water bottles were placed in respective cages on the alternate cage rack. Next, original cages were removed from the test room and bait dishes weighed (data recorded) and cleaned for the following day.

Residue Study

Two probes and a main residue study were conducted to assess bait consumptions/mortalities and to estimate whole-carcass Zn_3P_2/PH_3 residues in groups of voles fed test or control bait. Probes refer to initial pilot tests that involved development of GC-residue methodology with 2–3 voles each.

Voles: Three prairie voles were used in probe 1 (1♂ 2♀) and two meadow voles were used in probe 2 (1♂ 1♀). Twelve voles were used in the residue study—10 prairie voles (6♂ 4♀) and two meadow voles (1♂ 1♀).

Baits: Separate 500-g batches of 2.0% Zn_3P_2/2.0% Alcolec-S (wt/wt)/96% SRO groats (test) and 0.0% Zn_3P_2/2.0% Alcolec-S/98% SRO groats (control) were prepared. Samples of each bait were analyzed prior to use. The mean Zn_3P_2 concentration of test bait was 2.0% (±0.1); the control bait did not contain Zn_3P_2 at or above the Method Limit of Detection (MLOD) —<0.003%.

Materials: Approximately 15 g quantities of test or control bait were fed to each vole using the dish, cage insert, and other items described for the Acceptance Test.

Three LN_2 cryobiological storage/transport vessels were used: a Linde LR-17 (Union Carbide Corp., Danbury, CT), a MVE LAB1O, and a MVE LAB20 (Minnesota Valley Engineering, Inc., Minneapolis, MN). The Linde LR-17 and MVE LAB20 Dewars® served as carcass storage units, and the MVE LAB1O was used for LN_2 transport/replenishment.

Special 16 × 20.25 cm cryogenic bags (KAPAK Corp., Minneapolis, MN) were used to hold each carcass while in LN_2. Bags were sealed using a Mail Lite Heat Seal (Sealed Air Corp., Fair Lawn, NJ). Vole identification numbers were perforated into the "sealed area" of respective bags using a "tattoo outfit" having 3/8-inch digits (Stone Manufacturing and Supply Co, Kansas City, MO).

Procedures: The three prairie and two meadow voles chosen for use in probes 1 and 2 had averaged <10% intake of their body weight in control bait during the Acceptance Test. Voles that consumed ≥10% of body weight in control bait during the Acceptance Test were retained for the main residue study.

The 12 voles in the residue study were rank ordered by body weight. Four voles were studied daily; two of these were drawn randomly from the six voles weighing above and below the median weight, respectively. Three voles were fed test and one was fed control bait on days 1 and 2, with two test- and two control-bait voles studied on day 3.

Bait presentation was described previously. Pharmacotoxic sign observations were conducted every 15 min. Upon death, each test-bait vole carcass was removed from the cage and weighed. Each carcass was placed into a 500 ml disposable plastic beaker filled with LN_2 <5 min after death. Because PH_3 has a melting point of −133°C, carcasses were stored in LN_2 (−198°C) to keep PH_3 in the solid state until residue analysis (tissue temperatures did not exceed −180°C during preparation), eliminating the possibility of PH_3 loss through volatilization. After 5–10 min submersion in LN_2, each carcass was removed and sealed individually in a cryogenic storage bag. Bait consumption was then recorded.

Following the death of each day's "longest surviving" test-bait vole, the control-bait vole(s) was sacrificed by carbon dioxide (CO_2) exposure. Carcasses were stored in LN_2. Voles were analyzed for Zn_3P_2/PH_3 residues within 24 h of death.

Residue Determinations: Two measures of Zn_3P_2/PH_3 were used to estimate whole-carcass residues—available carcass PH_3 (*i.e.*, residual stomach-acid/hydrolyzed Zn_3P_2 available within carcass) and sulfuric-acid-(H_2SO_4)/released PH_3 (*i.e.*, undigested carcass Zn_3P_2 hydrolyzed during chemical analysis). Analytical methods developed during these studies were not characterized for bias or recovery.

Available carcass PH_3 was obtained by removing each carcass from the LN_2 and pulverizing it in a special stainless steel device. The device was a cylinder, 25.7 cm high (79.4 mm ID, 87.4 mm OD) comprised of a detachable lower cup (5.6 cm tall, 83.4 mm OD) which inserted/locked via a bayonet fitting into an upper cylinder (20.1 cm high).

Prior to use, the pulverizing device was partially submerged in a insulated reservoir filled with LN_2 (all objects that contacted vole tissue were at cryogenic temperatures). When the device was cooled to −198°C (verified by digital thermometer), each carcass was taken from LN_2 storage, placed immediately into the cylinder, and pulverized by repeated piston-like strokes of a metal bar (2.5 cm × 40.6 cm)

[1]Reference to trade names does not imply endorsement by the U.S. Government.

Table 1. Mean (±SD) body weight (at death), bait consumption, Zn_3P_2 intake, Zn_3P_2/body weight dose, and time to death for voles in probe 1, probe 2, and residue study

Test condition	Body weight (g)	Bait intake (g)	Zn_3P_2 intake[a] (mg)	Zn_3P_2 dose (mg/kg)	Time to death[b] (h:min)
Probe 1[c]	35.2	0.36	7.3	212.8	09:09
(n = 3)	(±11.5)	(±0.08)	(±1.6)	(±28.8)	(±00:06)
Probe 2[c]	44.8	0.35	7.0	157.0	09:12
(n = 2)	(±4.7)	(±0.01)	(±0.3)	(±10.3)	(±02:46)
Study					
Test	25.4	0.17	3.4	134.2	06:22
(n = 8)	(±3.3)	(±0.05)	(±1.1)	(±42.0)	(±02:52)
Control	33.3	1.17	—[d]	—[d]	—[d]
(n = 4)	(±14.2)	(±0.37)			

[a]Calculation of Zn_3P_2 intake based on 2% of bait consumption
[b]Time-to-death data based upon time-of-bait presentation, not initial consumption, for individual voles
[c]All voles in probes 1 and 2 were fed only test bait
[d]Euthanized shortly after the longest surviving test-bait vole died each day

for 5 min. The cylinder mouth was covered with a circular piece of semi-flexible plastic having a hole cut in the center to accommodate the bar. Next, the bar and cylinder wall were scraped to collect tissue residues, the cup was detached, and the powdered tissue transferred to a 1000-ml Erlenmeyer flask.

A thin layer of ultra-cold, powdered tissue was spread evenly over the flask bottom, and the flask was sealed immediately with a rubber septum. Flask contents were equilibrated at room temperature for at least 90 min, ensuring the passive diffusion and stabilization of PH_3 from the tissue into the flask headspace. Using a 10 μL Hamilton® glass syringe, 5 μL of headspace gas was sampled and injected into a Hewlett-Packard 5880 GC equipped with a flame photometric detector (phosphorus mode).

For the residue study, estimation of carcass PH_3 was made possible by a modification of the described procedure. Following the ≥90-min equilibration period, flask headspace pressure was measured in each of the specimen flasks using a digital pressure gauge (0.2–50.8 psi range). The pressure in all specimen and standard flasks was then increased (by injection of compressed air) to match the highest pressure observed. Five calibration standards ranging in concentration from 0.1 to 1.2 mg Zn_3P_2/flask were prepared fresh daily. All standards and samples were analyzed in duplicate. Standards-fitted linear regressions ($\bar{x}$ $r^2 = 0.999$, n = 3) were used to estimate headspace PH_3.

The H_2SO_4-released PH_3 (carcass Zn_3P_2) was measured immediately after the carcass-PH_3 analyses. Each specimen flask was opened (equalizing pressures), and 100 ml of 30% H_2SO_4 (vol/vol) was added. The flask was resealed, shaken for 90 min, and the headspace sampled for PH_3. No excessive headspace pressures were noted. Standards were prepared fresh daily at concentrations of ~0.5, 2.0, and 8.0 mg Zn_3P_2/flask. Flasks were analyzed and quantified using standards-fitted regression ($\bar{x}$ $r^2 = 0.999$, n = 3); standard/sample duplicates for analyses varied an average of 1.93(±1.9)%. Prior tests showed no hydrolysis of Zn_3P_2 in room-temperature, non-acidified homogenates, indicating that the two analyses were functionally distinct.

Results

Acceptance Test

Acceptance of the control bait was deemed satisfactory for conduct of the acute, single-exposure, probes/residue study using test bait. Mean daily consumption equaled 2.5 (±0.9), 3.0 (±0.9), and 2.8 (±0.8) g for days 1, 2, and 3, respectively (≥10.6% of the voles' body weights). A 3 (day) × 27 (vole) one-way repeated measures analysis of variance (Winer 1971) yielded no significant ingestion difference among days ($F_{2,52} = 1.27$, NS)—voles displayed little "learned acceptance/rejection of bait" across days. A repeated measures t-test (Winer 1971) showed that the voles lost a mean (±SD) 1.3 (±2.4) g or 5% weight when fed only SRO groats for three days ($t_{26} = 2.62$, $p < 0.05$).

Probes and Residue Study

Pharmacotoxic Signs: Control-bait voles were more active and ate bait more frequently than test voles; no unusual or pronounced postural/respiratory signs were evident for the controls.

Test-bait voles were usually active only within the first h after bait presentation. Subsequently, these voles were calm, lethargic, and huddled (*i.e.*, sitting with head forward and jaws touching front paws or cage insert) for the main portion of the post-bait period. "Pronounced signs" of toxicosis were not observed until ≤ 2 h preceding death; these included: prostrate on cage floor, irritability to stimuli, tonic movements (muscle extensions), ataxia (uncoordinated movements), apnea (interruption of respiration), dyspnea (shortness of breath), and gasps (aperiodic deep inspirations). For example, 4 of the 5 voles in the probes ate test bait within 18–40 min of initial presentation, then became "lethargic" and "calm" by 120 min—signs that persisted until ~1 h preceding death when apnea, dyspnea, and tonic movements were evident.

Bait Consumption/Mortality: Table 1 presents body weight, bait intake, dose, and time-to-death statistics for the voles.

Mean consumption of 2% Zn_3P_2/SRO groats by test voles was about a third [0.36 g (±0.08)] that of 0.0% Zn_3P_2/SRO groats by control voles [1.17 g (±0.37)]. The heavier voles in the probes consumed greater amounts of test bait than the lighter voles in the residue study. Minimum and maximum Zn_3P_2 intakes observed for specific voles were 2.0 and 8.2 mg,

Table 2. Mean (±SD; min-max) residue estimates for carcass PH_3, carcass PH_3 as percent of ingested Zn_3P_2, carcass Zn_3P_2, and carcass Zn_3P_2 as percent of ingested Zn_3P_2 of voles in the residue study[a]

Test condition	Carcass PH_3 (μg)	Carcass PH_3 as ingested Zn_3P_2 (%)	Carcass Zn_3P_2 (mg)[b]	Carcass Zn_3P_2 as ingested Zn_3P_2 (%)
Test (n = 8)	10.6[c] (±9.8; 0.5–21.0)	3.3 (±2.1; 0.2–6.0)	1.23 (±0.57; 0.8–2.2)	36.2 (±13.9; 19–58)
Control (n = 4)	NE[d]	—	≤MLOD[d]	—

[a] Data represent experimental values; the chemical analytical method was not validated
[b] Based on H_2SO_4-released PH_3
[c] One missing data value — leakage of flask septum
[d] Not estimated (NE); method limit of detection (MLOD) = 0.006 mg Zn_3P_2

respectively, whereas, Zn_3P_2 doses averaged ≥ 134.2 mg/kg (min-max 79.2–243.2 mg/kg).

All voles fed the Zn_3P_2 bait died, while none of the animals fed control bait died from bait ingestion (euthanized). Mean (±SD) times to death of the voles in probe 1 [≥ 09:08 (±00:06) h:min] and probe 2 [≥ 09:12 (±02:46) h:min] were longer than those of voles in the residue study [≥ 06:22 (±02:52) h:min]—heavier voles. Minimum-maximum times to death due to ingestion of Zn_3P_2 equaled 3 h, 42 min and 11 h, 52 min, respectively.

Zn_3P_2/PH_3 Whole-Carcass Residues: Table 2 presents residue statistics for the voles.

For the residue study, mean carcass Zn_3P_2 and carcass PH_3 were 1.23 mg (± 0.57; min-max = 0.48–2.20) and 10.6 μg (± 9.8; min-max = 0.5–21.0), respectively. The values of carcass Zn_3P_2 and PH_3 (as hydrolyzed Zn_3P_2) detected as a percentage of ingested Zn_3P_2 averaged 36.2% (± 13.9) and 1.3% (± 1.3), respectively, with ranges of 6-69% and 0.05–3.5%. Specimen PH_3 values were highly variable and very low relative to carcass Zn_3P_2. All Zn_3P_2 values for control-bait voles were <MLOD (4 ppb Zn_3P_2).

Regression Analyses: Linear regression equations were computed/plotted for selected pairs of dependent/regressor variables using PROC REG software (*SAS Institute* 1987).

Variations in body weight were predictive of bait consumption ($r^2 = 0.64$, intercept = −0.05, slope = 0.01 , $p \leq 0.001$) and carcass Zn_3P_2 ($r^2 = 0.60$, intercept = −1.67, slope = 0.11, $p \leq 0.002$). Because Zn_3P_2 intake was calculated as 2% of bait consumption (a direct transformation), Zn_3P_2 intake also yielded an equivalent regression with body weight ($r^2 = 0.64$,intercept = −0.94, slope = 0.19, $p \leq 0.001$). Test-bait consumption also accounted for significant statistical variation in carcass Zn_3P_2 [$r^2 = 0.32$, intercept = 0.06, slope = 6.89, $p \leq 0.043$], and as before, an equivalent regression was noted for Zn_3P_2 intake ($r^2 = 0.32$, intercept = 0.06, slope = 0.34, $p \leq 0.043$).

Sizable unexplained variation characterized the regressions involving the time-to-death variable. Neither body weight ($r^2 = 0.25$, $p \geq 0.08$), consumption/Zn_3P_2 intake ($r^2 = 0.21$, $p \geq 0.11$), nor carcass Zn_3P_2 ($r^2 = 0.05$, $p \geq 0.47$) predicted significant variation in the time to death of voles. These regressions were not different from zero (no linear fit).

Figure 1 presents scatterplots of three pairs of ingestion/weight/residue variables. These plots show that: (A) as expected, heavier voles consumed greater amounts of test bait and Zn_3P_2, (B) body weight was an important regressor of carcass Zn_3P_2, and (C) for 9 of 13 carcasses, only ~25–50% of calculated Zn_3P_2 intakes were accounted for during chemical analysis.

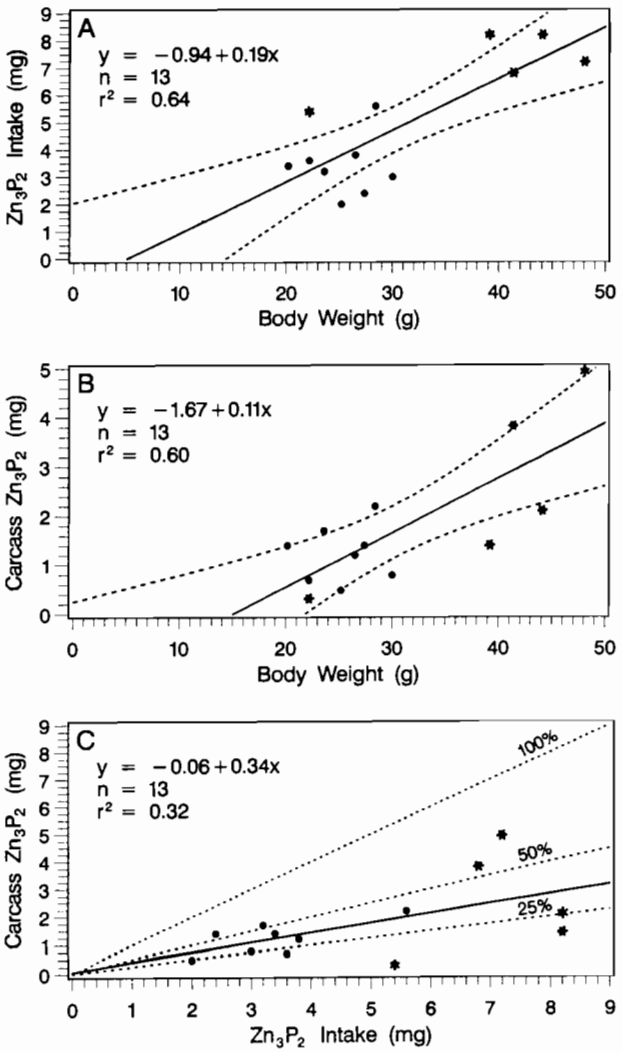

Fig. 1. Scatterplots with "best fit" regression lines between three pairs of ingestion/weight/residue variables in voles: (A) Zn_3P_2 intake (mg) and body weight (g), (B) carcass Zn_3P_2 (mg) and body weight (g), and (C) carcass Zn_3P_2 (mg) and Zn_3P_2 intake (mg). Note that the symbol "*" denotes data values obtained for carcasses in probes 1 and 2; the dashed lines in A and B represent 95% confidence intervals, but in C provide 25%, 50%, and 100% projections of the Zn_3P_2 intake variable

Discussion

The results show that ~25–50% of ingested Zn_3P_2 is accounted for, using the current whole-carcass residue procedure. Interestingly, using a colorimetric method of PH_3 absorption, Tkadlec and Rychnovsky (1990) detected only ~58% (±25%) of ingested Zn_3P_2 in the GI tracts of Zn_3P_2-killed voles, with only 0.3% (±0.3%) of ingested Zn_3P_2 found in the remainder of carcasses. Bias and recovery of the analytical methods, however, was not assessed in either study.

The cryogenic technique permitted the capture and subsequent detection of carcass PH_3 residues, but analytical quantitation of PH_3 recovery proved difficult. Increased internal flask pressures resulted from the rapid room-temperature-induced expansion of supercooled and condensed air associated with the introduction of cryogenically-processed tissue. This problem was controlled by equalization of all flask pressures, and could be avoided by using a pressure-independent gas sampling system.

Phosphine is highly toxic (*e.g.*, LC = 0.75 mg/L in mice, LC = 0.07-3.5 mg/L in cats). This gas is highly reactive in the presence of plant and animal tissues, and varying degrees of recovery have been reported from plant products (Berck and Gunther 1970; Hilton and Mee 1972). Whether present in the animal at the time of death or produced by Zn_3P_2 hydrolysis during analysis, PH_3 is likely to react with surrounding tissue. Reduced recovery of H_2SO_4-released PH_3 from animal tissue was noted by Terzić (1984), and subsequent work in our laboratory has shown PH_3 (from H_2SO_4-hydrolyzed Zn_3P_2) recovery from homogenized vole tissue to be <100% and highly variable.

Undigested Zn_3P_2 poses the main hazard to predators and scavengers consuming poisoned voles (*i.e.*, a primary, not secondary, type hazard). Of course, potential hazards are dependent upon numerous factors: (1) the amount of undigested Zn_3P_2 remaining in the dosed animal at the time of death (*i.e.*, primary hazard), (2) the portions of the carcass ingested or rejected (*i.e.*, consumption of vole GI tracts should yield the greatest within-carcass loads of active Zn_3P_2), (3) the climatological conditions affecting carcass decomposition (*i.e.*, palatability of carcass), and (4) the accessibility of the carcass (*i.e.*, exposed/unexposed to the forager).

Acknowledgments. We thank Stanley Gaddis for technical assistance, Melvyn Garrison and Jerry Roberts for bait formulations, Steve Griener and Rori Craver for analyses of Zn_3P_2 and baits, Bruce Wunder for vole gender/species identifications, Paige Groninger for regression analyses and Figure 1, and Larry Clark and Edward Schafer, Jr. for helpful critiques of the manuscript.

References

Bell HB (1972) The hazards of secondary poisoning from zinc phosphide to selected vertebrate species. Unpubl MS thesis, University of Tennessee, Knoxville, TN, 33 pp

Bell HB, Dimmick RW (1975) Hazards to predators feeding on prairie voles killed with zinc phosphide. J Wildl Manage 39(4):816–819

Berck B, Gunther FA (1970) Rapid determinations of sorption affinity of phosphine by fumigation within a gas chromatographic column. J Agric Food Chem 18(1):143–147

Chan LTF, Crowley RJ, Delliou D, Geyer R. (1983) Phosphine analysis in *post-mortem* specimens following ingestion of aluminum phosphide. J Anal Toxicol 7(4):161–208

Childs JE, Ksiazek TG, Rollin PE, Krebs JW, Zaki S, Nichol ST, Peters CJ, Glass GE (1994) Hantaviruses and their rodent reservoirs in the United States. Proc Vertebr Pest Conf 16:188–191

Gratz NG (1973) A critical review of currently used single-dose rodenticides. Bull Wld Hlth Org 48:469–477

Hilton HW, Mee JML (1972) Studies with radioactive phosphine-^{32}P in sugarcane. J Agric Food Chem 20(2):334–336

Hood GA (1972) Zinc phosphide—A new look at an old rodenticide for field rodents. Proc Vertebr Pest Conf 5:85–92

Marsh RE (1988) Relevant characteristics of zinc phosphide as a rodenticide. Great Plains Wildl Dam Cont Wkshp Proc 8:70–74

Nowicki TW (1978) Gas-liquid chromatography and flame photometric detection of phosphine in wheat. J Assoc Offic Anal Chem 61(4):829–836

SAS Institute (1987) PROC REG. In: Parker JC (ed) SAS/STAT™ Guide for Personal Computers (Version 6). SAS Institute, Inc, Cary, NC, pp 1–18

Tabata K (1986) Decomposition of the rodenticide Zn_3P_2 and its acute toxic symptoms in red-backed wood mice (*Apodus speciosus*)(in Japanese). J Jpn For Soc 68(1):26–31

Terzić Lj (1984) Spectrophotometric determination of zinc phosphide in technical products and tissues from poisoned animals. Acta Vet (Beograd) 34(2–3):97–103

Tkadlec E, Rychnovsky B (1990) Residues of Zn_3P_2 in the common vole (*Microtus arvalis*) and secondary poisoning hazards to predators. Folia Zool 39(2):147–156

Winer, BJ (1971) Statistical principles in experimental design (2nd Edition). McGraw-Hill, NY, pp 44–48, 261–283